PURIFICATION AND ACTIVITIES OF PURINE ENZYMES FROM VARIOUS TISSUES OF THE AMERICAN COCKROACH *PERIPLANETA AMERICANA* LINNAEUS. (ORTHOPTERA: BLATTIDAE)

Sally M. Cordero and Daniel Ludwig
Department of Biology, Fordham University, N. Y. 58.

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ABSTRACT

A study was made of the activities of adenosine deaminase, guanase, xanthine oxidase and uricase in homogenates of the foregut, midgut, hindgut, Malpighian tubules and leg muscle of the American cockroach, *Periplaneta americana.* Adenosine deaminase was found in all tissues except leg muscle. Its activity was higher in homogenates of the gut than in those of the Malpighian tubule. Guanase, uricase and xanthine oxidase were present in all tissues. Their activities were highest in the gut and Malpighian tubules, suggesting that these organs play an important role in purine metabolism.

A step in intermediary metabolism of nitrogenous compounds is deamination. An important enzyme in this reaction is adenosine deaminase which breaks down adenosine to inosine. This enzyme was detected by Lennox (1940) in the gut and skeletal muscle of the dipteran *Lucilia cuprina.* Guanine is deaminated to xanthine by guanase. This enzyme has been found by Florkin and Duchâteau (1943) in the water beetles *Hydrophilus* and *Dytiscus.* Lisa and Ludwig (1959) obtained it from fat bodies of the tropical cockroach *Leucophaea maderae.*

Another step in purine metabolism is oxidation. Hypoxanthine and xanthine are oxidized to uric acid by xanthine oxidase. Morgan (1926) found this enzyme in nymphs of various insects. Ross (1959) and Lisa and Ludwig (1959) found xanthine oxidase in the Japanese beetle *Popillia japonica* and the cockroach *Leucophaea maderae,* respectively. Dixon and Lemberg (1934) found that, in purified form, it does not oxidize combined purines. Other enzymes in crude tissue preparations liberate hypoxanthine so that oxidation can take place.

Uricase oxidizes uric acid to allantoin. Ross (1959) found it in newly laid eggs and in larvae of the Japanese beetle, *Popillia japonica,* but was unable to detect it in the prepupa, pupa, or

adult of this insect. Lisa and Ludwig (1959) isolated it from fat bodies of the tropical cockroach.

The present work was undertaken to determine the presence of some enzymes concerned with the intermediary purine metabolism in different tissues of the American cockroach, *Periplaneta americana.* A study was made of the activities of adenosine deaminase, guanase, xanthine oxidase, and uricase in homogenates of the foregut, midgut, hindgut, Malpighian tubules, and leg muscle. These enzymes were obtained in concentrated form and their activities studied.

Material and Methods

The cockroaches were reared in glass jars, at room temperature (about 25°C.). Laboratory food pellets and water were available at all times. Adults, two days after the last molt, were used in all experiments. The digestive tract was removed and subdivided into foregut, midgut and hindgut. Malpighian tubules and leg muscles were also removed. Each portion was cleaned of any debris, and washed several times with ice-cold insect saline solution (Ludwig, Tracey and Burns, 1957), and was studied separately. It was homogenized in the proper buffer and the homogenate was used at a concentration of 100 mg. of tissue per ml. of buffer. In each experiment, except xanthine oxidase determinations, 0.1 ml. of homogenate was used. Controls were treated the same as the reaction mixture, but for determinations of adenosine deaminase, guanase and uricase, 0.1 ml. of boiled homogenate was used, while KCN treated homogenates were used for xanthine oxidase studies.

Enzyme activities were measured with the Beckman DU spectrophotometer. The activity of adenosine deaminase was measured by the method of Kaplan (1955). It is based on the decrease in optical density at 265 mμ when adenosine is deaminated to inosine. Homogenates of the various tissues were made in 0.5 M phosphate buffer at a pH of 7.4. Determinations were made with 2.7 ml. phosphate buffer and 0.2 ml. of adenosine solution and 0.1 ml. homogenate in each of two silica cuvettes (the control contained boiled homogenate). Readings were made at 30 second intervals for 3.5 minutes. Adenosine deaminase from the midgut and hindgut was concentrated by the method of Kalckar (1947a) as modified by Kornberg and Pricer (1951), and the activities of these concentrates were determined.

Guanase activity was studied by the method of Roush and Norris (1950) and is based on the decrease in optical density at 245 mμ when guanine is deaminated to xanthine. Homogenates were made in 0.5 M tris buffer at a pH of 8.0. In each of two silica cuvettes, 2.7 ml. of tris buffer, 0.1 ml. of guanine, and 0.1 ml. of homogenate were placed. Readings were made at 30 second intervals for three minutes. Guanase was purified from the hindgut and Malpighian tubules by the method of Kalckar (1947b) and assayed by the method of Roush and Norris (1950).

The activity of uricase was studied by the method of Schneider and Hogenboom (1952). It is based on the decrease in optical density at 290 mμ when uric acid is oxidized to allantoin. Homogenates were prepared in 0.5 M phosphate buffer at a pH of 7.4. To each of two silica cuvettes, 2.6 ml. of water, 0.2 ml. of phosphate buffer, 0.1 ml. of uric acid, and 0.1 ml. of homogenate were added. Readings were made at one minute intervals for five minutes. Uricase was purified from the hindgut and Malpighian tubules according to the methods of Leone (1953) and of London and Hudson (1956). It was assayed by the method of Schneider and Hogenboom (1952).

The activity of xanthine oxidase was determined by the method of Horecker and Heppel (1949). It is based on the change in density at 550 mμ as cytochrome *c* is reduced by the oxidation of hypoxanthine to uric acid. Homogenates were made in 0.1 M phosphate buffer at a pH of 7.4. In each of two corex cuvettes were placed 2.0 ml. of phosphate buffer, 0.1 ml. of 2.5×10^{-4} M cytochrome *c*, 0.1 ml. of catalase, 0.1 ml. of bovine albumin, and 0.1 ml. of water. Then 0.4 ml. of fresh homogenate was added to the reaction mixture and 0.4 ml. of KCN treated homogenate to the control. Readings were taken at one minute intervals for 7 minutes after the mixtures were combined with 0.1 ml. of 0.05 M hypoxanthine. Then 1 mg. of solid $Na_2S_2O_4$ was added and a final reading was made. Calculation of enzyme activity were made using the formula of Horecker and Heppel 1949. Xanthine oxidase was concentrated by the method of Horecker and Heppel (1949) from the hindgut and Malpighian tubules and its activity measured.

Observations

Table I contains average values for ten determinations of the

activities of adenosine deaminase, guanase, and uricase in tissue homogenates and five determinations for concentrated adenosine deaminase, guanase, and uricase. For each enzyme, activity of the gut was greater the more posterior the region selected for the determination. The activity of adenosine deaminase was greatest in the hindgut. Malpighian tubules showed less activity than the gut, and no activity was found in the muscles. Guanase activity was detected in all the tissues studied. It was greatest in the

TABLE I.

Activities of adenosine deaminase, guanase, and uricase in various tissues of the cockroach. Activity is expressed as μg. of substrate converted by 1 ml. of homogenate per hour.

Tissue	Adenosine deaminase	Guanase	Uricase
Foregut	7.2	6.6	3.6
Midgut	7.4	11.6	19.2
Hindgut	7.5	12.2	33.6
Malpighian tubules	3.8	14.6	30.0
Leg muscle	0.0	11.0	6.0
Concentrated enzyme	8.1	22.4	43.2

TABLE II.

Activity of xanthine oxidase, expressed as moles of cytochrome *c* reduced per ml. of homogenate per hour.

Tissue	Activity
Foregut	0.189
Midgut	0.191
Hindgut	0.348
Malpighian tubule	0.702
Leg muscle	0.094
Concentrated enzyme	4.242

Malpighian tubules. Uricase activity was also found in all of the tissues studied but its activity was greatest in the hindgut. In each case, the concentrated enzyme showed an increased activity over that of the tissue homogenates.

The activities of xanthine oxidase in various tissues are given in Table II. The enzyme from the Malpighian tubules was most active. The concentrated enzyme showed a greatly increased activity over that of the tissue homogenate.

DISCUSSION

Brown and Farber (1936) assumed that deamination of adeno-

sine in the blow flies *Lucilia sericata* and *Calliphora erythrocephala* occurs mostly in the tissues of the hindgut and midgut. They observed that the addition of intestinal extract as a substrate has a short stimulating effect on deamination of breakdown products of the proteins. These end-products become substrates for deamination which may take place in the cells of the posterior segment of the midgut or in the hindgut. This observation is corroborated by the present work, since the deaminase was found most active in the tissues of the midgut or hindgut.

There is a possibility that guanine is converted to xanthine as soon as it is formed since guanine has not been detected in insect tissue. The present work indicates that the gut, Malpighian tubules and leg muscles are probable sites for this reaction. These results are in agreement with those of Anderson and Patton (1955) who also found guanase in the gut and fat body of *Periplaneta americana, Prodenia eridana* and *Tenebrio molitor*.

The distribution of uric acid in different insects has been fairly well established but its oxidation to allantoin has not been generally accepted. Investigators have indicated that the principal sites for this activity are the Malpighian tubules and the fat body. Brown (1938a and b) stated that the nitrogen metabolism in the blow fly, *Lucilia sericata,* is through uric acid which is present in various stages of the life cycle and is oxidized to allantoin except in the prepupa and pupa. This work was confirmed by Ross (1959) who detected uricase in the newly laid eggs and larvae of the Japanese beetle *Popillia japonica,* but was unable to find it in the prepupa, pupa or adult. Lisa and Ludwig (1959) studied uricase from the fat body of nymphs and adults of the cockroach, *Leucophaea maderae,* and found that its reaction is of the first order with a Michaelis constant of 1.7×10^{-5} moles per liter. This figure is in general agreement with that obtained for uricase in mammalian tissue. The present work is an extension of their findings since uricase was detected in all the tissues studied.

Allantoin, the product of uric acid oxidation, has been detected in other insect tissues. It was found in the wings of Pieridae by Schöpf (1939). Ludwig and Cullen (1956) found both uric acid and allantoin in the blood of the Japanese beetle. These findings were corroborated by Heller and Szarkowska (1957) who found

uric acid in the blood of the moths, *Celerio euphorbiae* and *Sphinx pinastri.* Leifert (1935) found uricase in the pupa of the moth, *Antheraea pernyi.* Rocco (1938) found it in the carnivorous beetles of the families Dytiscidae and Carabidae, and in various Orthoptera. Truskowski and Chajkinowa (1935) found it to be very active in the adult muscid flies but absent in the German cockroach *Blatella germanica,* in the beetle *Melolontha,* in the bee *Apis mellifica* and in aphids.

Very little is known of the organs concerned with the formation of nitrogenous waste products, or of their chemistry. Although it has been stated by Leifert (1935), and by Anderson and Patton (1955), that the site for purine metabolism is the fat body, the present work indicates that the gut and the Malpighian tubules also play an important role since all of the enzymes studied were detected in these tissues.

Summary

1. Adenosine deaminase from the foregut deaminated 7.2, the midgut 7.4, the hindgut 7.5, and the Malpighian tubules 3.8 micrograms of adenosine per ml. of 0.1 per cent homogenate per hour. No activity was found in the muscle. The activity value for concentrated enzyme was 8.1 micrograms per ml. of enzyme preparation per hour. Corresponding values for guanase were 6.6, 11.2, 12.2 and 14.6, respectively. This enzyme from muscle converted 11.0 micrograms of guanine per ml. of 0.1 per cent homogenate per hour. The activity value for purified enzyme was 22.4.

2. Uricase from the foregut oxidized 3.6, the midgut 19.2, the hindgut 33.6, the Malpighian tubules 30.0 and the leg muscles 6.0 micrograms uric acid per ml. of 0.1 per cent homogenate per hour. The corresponding figure for the concentrated enzyme was 43.2.

3. The activity of xanthine oxidase, expressed in moles of cytochrome *c* reduced per ml. per hour, were foregut 0.189, midgut 0.191, hindgut 0.348, Malpighian tubules 0.702, and leg muscle 0.094. The corresponding value for the purified enzyme was 4.242.

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UNDESCRIBED SPECIES OF CRANE-FLIES FROM THE HIMALAYA MOUNTAINS (DIPTERA: TIPULIDAE), VIII*

BY CHARLES P. ALEXANDER

AMHERST, MASSACHUSETTS

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ABSTRACT

The following new species are defined: *Gonomyia* (*Progonomyia*) *bisiculifera; G.* (*P.*) *protensa; G.* (*Gonomyia*) *tanaocantha,* from Sikkim; *G.* (*Lipophleps*) *kama; G.* (*L.*) *varsha* from Kumaon and West Bengal, and *G.* (*L.*) *mizoensis* from the Lushai Hills, Assam.

The preceding part under this general title was published in the *Journal of the New York Entomological Society, 70:* 10–16; 1962. As was the case in all recent parts the materials were included in vast series of these flies collected by Dr. Fernand Schmid, collections so rich as to have virtually doubled the number of species of these flies previously known from India. My deep thanks again are extended to Dr. Schmid for the privilege of studying this important series. The types of the new species are in my personal collection of Tipulidae.

Gonomyia (Progonomyia) bisiculifera new species

Size medium (wing of male about 5.5 mm.); mesonotal praescutum and scutum polished black, posterior sclerites and the pleura more pruinose, scutellum darkened; knobs of halteres whitened; legs black, fore femora narrowly yellowed at bases; wings grayish white, beyond cord more darkened,

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* Contribution from the Entomological Laboratory, University of Massachusetts.